

Tribolium castaneum



All predicted exons (71,788) from the 'official gene set' of the recently sequenced genome of the red flour beetle, *Tribolium castaneum*, were used to calculate independent designs of long dsRNAs for the *Tribolium* genome (exons longer 560 nt were additionally splitted in two sequences, resulting in overall 80,671 input sequences). *Tribolium* has become an important model organism for developmental and evolutionary studies and is suitable for RNAi by injection of long dsRNAs. Information on predicted genes and sequences was obtained from [BeetleBase](#) that integrates sequence annotations for *Tribolium*.

NEXT-RNAi results

NEXT-RNAi HTML outputs are available [here](#)

Overall 71,293 designs were obtained, covering 99.4% of the genome. 92.9% of all genes are covered by at least one design that does not show homology of 19 nt or longer to any other gene. 83.2% of all genes are additionally covered by at least one second, independent design.

Input files and settings used

Input FASTA file

[beetlebase_exons_split.zip](#) (6.6MB) containing exon target sequences as input file (`-i` input) (parsed from *Tribolium* GFF files).

```
>TC016216-RA-E3
GGCACGCACATCTCAGTCCCTGCCAACGGAAATGATTTTGCACATTTTGCGGTGGGTGGTGAGTGCGGAG
CTGGATTTGAGGTCGCTGGAGATGTTTTCGATGGTGTGTGCGGGCTTTTATTTGTGCGCACGTGACCCG
GAGGTGTGGCGGTTGGCGTGCCTGAG
>TC008956-RA-E3_1
GCTGCACTTCCGACCGAAGACGCCGCCCGCCCGACGAAAGAGTCCCCGTGCAGTTTGCTCCCGTTCAGCGA
GAGTTATTTTCGTCCCCGGTGCCAACATGTCGTTCTCAATCGAGGTCCAGTTCGTTGGAAAACGCCAAGTAC
ACTCCTGGACACTTCCGCTTCTTAACCGATCCATTCACGTTTGATAATTTGGACAGTATAGAAGACGT
ACCGGTAACCGACACGCGGCAATCCAGTGATAAAAATCGTGCAAACGGGCATTAACCTGAACGTTAATGT
CGGTCTCAATACGCTTACTCCTAGTGTTAACG
>TC008956-RA-E3_2
ATAATAGTAGTAGTGTFTTTTCAAATAGTAACGCGAACCTCACTGATAGTATCAAGCTCGAGGATGCGA
CGTCATCGTCGACGTCGGACGAAAACGACGAAAAGACTATCGGATGTTTCGAGGTTTACGTGTCCGGATT
TGGGCGTCACGAAGAAAAATCAAACGTCCAAAACGCAAACCGTTACGAGTACCTTGACTAGTCCCTAAGT
TTAATAGTGTCAACGCCGTGTCTGAGGGATGGAGTAGGAGTACAGCCACCGAAGGGCCCTTGACTAACAC
CCAACGGACTGCTCTCGCAGTTTGGCCTGGATG
```

Targetgroup file (tab-delimited)

[beetlebase_targetgroups.tab](#) (732KB) defining which BeetleBase transcripts belong to the same gene (headers Target and TargetGroup) ([TARGETGROUPS](#) option)

Target	TargetGroup	TxnName	GeneName
TC004355-RA	TC004355	TC004355-RA	GLEAN_04355
TC004356-RA	TC004356	TC004356-RA	GLEAN_04356
TC004357-RA	TC004357	TC004357-RA	GLEAN_04357
TC004358-RA	TC004358	TC004358-RA	GLEAN_04358
TC004359-RA	TC004359	TC004359-RA	GLEAN_04359
TC004360-RA	TC004360	TC004360-RA	GLEAN_04360

Bowtie database/index for off-target evaluation

Bowtie database/index containing annotated BeetleBase transcripts for specificity calculations (-d input):
[beetlebase_transcript.tar.gz \(26MB\)](#)

Bowtie database/index for mapping of reagents

The Bowtie database/index ([GENOME BOWTIE](#) option) for mapping of reagents to the *Tribolium* genome:
[beetlebase_genome.tar.gz \(148MB\)](#)
Bowtie indexes for genomes are also available through the [Bowtie webpage](#).

FASTA file for homology evaluation

Transcriptome FASTA file to evaluate the homology of the designs using Blast ([HOMOLOGY](#) option):
[beetlebase_transcript.zip \(6.2MB\)](#)

Design criteria

Start of program

```
perl nextrnai.pl -i beetlebase_exons_split.fa -s 2500 -r d -d  
beetlebase_transcript  
-e NO -o options.txt -n Tcas_3.0
```

Descriptions for start parameters used are available [here](#).

Options file

```
DESIGNWINDOW=80,250
DESIGNNUM=50
OUTPUTNUM=1
SIRNALLENGTH=19
EFFICIENCY=SIR,0
REDESIGN=ON
BOWTIE=/usr/bin/
TARGETGROUPS=beetlebase_targetgroups.tab
PRIMER3=/usr/bin/
GENOMBOWTIE=beetlebase_genome
GFF=GFF3
GBROWSEBASE=http://www.dkfz.de/signaling/cgi-bin/gbrowse_img/beetlebase/
GBROWSETRACK=GENE+TXN
AFF=YES
LOWCOMPEVAL=/usr/bin/
CANEVAL=6
HOMOLOGY=/usr/bin/,beetlebase_transcript.fa,1e-10
RANKD=SPEC
```

Descriptions for all options used are available [here](#).